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Significance of microvessel density in prostate cancer core biopsy

Značaj gustine krvnih sudova u biopsijama karcinoma prostate

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Abstract

Background/Aim. In prostate tumors, angiogenesis, measured as microvessel density, is associated with tumor stage and Gleason score. The aim of this study was determine neovascularization of prostatic adenocarcinomas in core biopsies and corresponding prostatectomies. **Methods.** The study population included 61 patients who underwent radical prostatectomy (RP) for localized prostate carcinoma patients and did not receive chemohormonal, or radiation therapy before surgery. Tumor blocks were immunostained using the endothelial-specific antibody CD31 and subsequently evaluated at × 400 magnification in both biopsies and corresponding prostatectomies. **Results.** When comparing microvessel density in core biopsies and corresponding prostatectomies, no statistically significant difference was found ($p > 0.1$). A statistically significant positive correlation was found when determining correlation between microvessel density (as linear and categorical variable, i.e. with the cut-off value of 48) that was associated with the Gleason score ($p < 0.05$) and tumor stage ($p < 0.0001$). There was no correlation between microvessel density and preoperative values of serum prostate-specific antigen (PSA) ($p > 0.1$). **Conclusion.** Microvessel density can be reliably applied to needle prostate biopsy specimens. Quantification of the microvascular density in biopsies is an accurate pre-operative predictor of tumor stage, discriminating between organ-confined and organ-extending neoplasms.

Key words:
prostatic neoplasms; biopsy, fine-needle; prostatectomy; immunohistochemistry; neovascularization, pathologic.

Apstrakt

Uvod/Cilj. U karcinomima prostate, angiogeneza određena merenjem gustine krvnih sudova, povezana je sa stadijumom tumora i Gleason skorom. Cilj ovog istraživanja bio je proučavanje neovaskularizacije adenokarcinoma prostate u uzorcima biopsije iglom i odgovarajućih prostatektomija. **Metode.** U naše istraživanje bio je uključen 61 bolesnik kojima je urađena radikalna prostatektomija (RP) na osnovu kliničke procene da se radi o lokalno ograničenom karcinomu prostate, koji preoperativno nisu primili nikakvu hemio-, hormonalnu ili zračnu terapiju. Tumorsko tkivo je analizirano primenom imunohistohemijskog markera, endotel-specifičnog antitela CD31, koje je zatim procenjivano na mikroskopskom uveličanju × 400 u uzorcima biopsija iglom i tkiva dobijenih nakon RP. **Rezultati.** Prilikom poređenja vrednosti gustine krvnih sudova određenih na biopsijama prostate uzetih iglom sa gustinom krvnih sudova odgovarajućih RP nije nađena statistički značajna razlika ($p > 0,1$). Statistički značajna pozitivna korelacija nađena je prilikom određivanja povezanosti gustine krvnih sudova (kao linearne i kategorijalne varijable sa ograničenom vrednošću 48) i Gleason skora ($p < 0,05$), kao i stadijuma bolesti ($p < 0,0001$). Statistički značajna povezanost nije utvrđena između gustine krvnih sudova i predoperativnih vrednosti serumskog prostatičnog specifičnog antigena (PSA) ($p > 0,1$). **Zaključak.** Određivanje gustine krvnih sudova može se pouzdano koristiti za uzorke prostate dobijene biopsijom iglom. Kvantifikacija gustine krvnih sudova u biopsijama iglom tačan je i nezavisan predoperativni prediktor stadijuma tumora (lokalno ograničen karcinom prostate u odnosu na lokalno proširenu bolest).

Ključne reči:
prostata, neoplazme; biopsija tankom iglom; prostatektomija; imunohistohemija; neovaskularizacija, patološka.

Introduction

The incidence of prostate cancer (PC) is on an exceptional increase in the whole world, thanks to early detection programs that include digital rectal examination, determining of serum prostate-specific antigen (sPSA), transrectal ultrasonography and needle biopsy of the prostate [1, 2]. This is a heterogeneous disease with unpredictable clinical flow from a relatively indolent disease to an aggressive form with rapid metastatic spreading of the disease and fatal outcome. Unfor-

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tunately, there are still no parameters that can be safely used to foresee whether it is a locally non-invasive prostate cancer (pT2) or invasive and metastasis expanded neoplasm (pT3). According to data from different researches published so far, PC in 24–60% patients was clinically under-staged before surgery, whereas in 8–45% of cases it was over-staged [3–9]. Potential biomarkers are still being researched as well as different methods of diagnostics, which could improve detection, preoperative grading and staging systems for prostate cancer in order to get a clearer picture about possibilities and risk of the application of adequate therapy procedures for each individual patient. Angiogenesis (neovascularization) is the process of creation of new functional capillary microvessels from the already existing vascular network. Vascularization of the primary tumor results in an expanded growth and the tumor then gets metastatic potential, whereas development of microvessels is necessary for growth of distant metastatic tumor hotspot [10].

Tumor microvessels are not the same as the microvessels of normal tissue, they are heterogeneous in terms of organization, function and structure [11]. Angiogenesis is present in all the tumors, but with characteristic and significant differences between different types of tumors (the biggest intensity of angiogenesis was found with glioblastoma, followed by renal cell carcinoma, colorectal cancer, breast cancer, lung cancer, PC), but inside every individual type of tumor in different patients – individual tumors are well vascularized, while the others are poorly vascularlized [12]. The prognostic value of microvessel density (MVD), as a measure of tumor angiogenesis, is still unclear in the PC, particularly on samples of needle prostate biopsies [13–15]. Thus, the aim of this study was to determine prognostic significance of MVD, as a stage predictor in prostatic carcinomas in core biopsies (CB) and corresponding prostatectomies.

Methods

This retrospective study included 61 previously untreated patients with prostatic adenocarcinoma admitted with elevated serum PSA levels at the Clinic of Urology, Clinical Center of Vojvodina, in the period 2005–2006. All the patients underwent systematic sextant transrectal ultrasonography-guided core biopsies performed with an 18-gauge automated spring-loaded biopsy gun. The diagnosis of prostatic carcinoma in needle biopsies was followed by radical retropubic prostatectomy (RP) with bilateral pelvic lymphadenectomy. All tumors were primary diagnosed without previous therapy and none of the patients had clinical evidence of metastasis prior to surgery. Tumor grading on needle biopsies and prostatectomy specimens were undertaken according to Gleason. The final pathological stage on the whole mount prostatectomy specimens was determined according to the tumor-nodus-metastasis (TNM) system.

From these 61 patients, a total of 366 core biopsies were available, out of which 254 contained carcinomatous tissue (median 2; range 1–6 *per* case). These 254 biopsies were analyzed and only those containing at least two microscopic fields of neoplastic glands at x400 magnification were selected for determination of MVD. Insufficient tumor tissue was found in 55 core biopsies, and these biopsies were excluded. Finally, analyses were performed on 199 core biopsies. All RP specimens from 61 patients were evaluated in a standard fashion. Surgical margin (SM) sections from the apex and base were taken as shaved margins. Extraprostatic extension (EPE) was diagnosed if tumor was seen in the periprostatic soft tissue or was seen penetrating through a fibromuscular capsule and coming out on the other side. The seminal vesicles (SVs) were evaluated at the junction where they enter the prostate gland. All pelvic lymph nodes were evaluated for the presence of metastatic disease. All the cases were assigned with a Gleason sum (GS). After review of each case, the blocks with the highest GS and greatest density of tumor and those containing at least two microscopic fields of neoplastic glands at ×400 magnification were selected for immunohistochemical staining. The study excluded the patients who had received prostate-related therapy before RP, including androgen deprivation therapy, chemotherapy, radiation therapy, or other therapy. It also excluded the patients in whom there was no cancer remaining in the needle biopsy tissue to perform MVD analysis, as well as matched totally embedded RP specimens.

Serum PSA concentration was determined before RP and analyzed as a continuous and categorical variable with the cut-off value 10 ng/mL and 20 ng/mL.

Immunohistochemistry

Tumors from 61 patients – 199 core biopsies with sufficient tumor areas, as well as sections from 61 selected tissue blocks of corresponding RP were analyzed by immunohistochemistry. Routine formalin-fixed, paraffin-embedded 3–4-µm-thick sections from each patient were attached to silanized slides, sequentially deparaffinized and rehydrated. Access to tissue antigen sites for antibody attachment was enhanced by microwaving slides which were treated by citrate buffer for 20 minutes. Detection of microvessels was performed using a monoclonal antibody against the CD31 antigen (clone JC/70A; Dako, Glostrup, Denmark). Dilution of the primary antibody was 1 : 40 in Tris buffered saline (TBS) / 1% BSA / 1% human serum and were incubated for 30 minutes. The EnVision technique and development with the chromogen 3,3´-diaminobenzidine tetrachloride (DAB) was used for visualization. Sections were lightly counterstained with hematoxilyn. Intense cytoplasmic immunoreactivity was observed in endothelial cells of small, medium-sized and large blood vessels in all study specimens. Normal prostate biopsy tissue served as a negative control after deletion of the primary antibody step and substitution of buffer during each run to suppress microvessel staining.

Determination of microvessel density

MVD was determined by light microscopy analysis for the areas of the tumor containing the most capillaries and small venules (microvessels, neovascular "hot spots") using the counting method introduced by Weidner and modified by Rogatsch [13, 16–18]. Prostate cancers are multifocal and heterogeneous in their MVD. The tumor area in CB and RP specimens containing the maximum number of descrete (brown) microvessels staining for CD31 was identified by scanning at low power (×40

and ×100) [13, 16, 17]. These areas were most frequent at the margins of carcinoma. After identification of the three areas of highest neovascularization, individual microvessels were counted at ×400 magnification, where one field is equivalent to 0.19 mm² representative ×400 high power fields (Olympus BH-2 microscope, Olympus Optical Co. Ltd., Japan) [18]. Both isolated immunoreactive brown-staining endothelial cells and endothelial cell clusters, separate from adjacent microvessels clearly, tumor cells and connective-tissue elements, were considered countable vessels. Vessel lumens do not need to be considered as a microvessel and red blood cells were not used to define a vessel lumen. Exclusion of occasional immunoreactive macrophages and plasma cells was based on their morphological appearance [13, 16, 17]. The highest readings in CB and corresponding prostatectomy were expressed as the highest number of microvessels identified within any single ×400 field. An average of multiple fields was not used. Assessment of MVD was done without knowledge of any clinicopathological data. MVD within normal prostate tissue and hyperplastic nodules served as internal control.

Statistics

The correlation of MVD (in a categorical and continuous fashion) in biopsies and corresponding prostatectomies was calculated using the MANOVA. Microvascular counts in organ confined (pT2) *versus* organ-extending tumors (pT3) were compared with MANOVA and $\chi 2$ test. To determine the relationship between tumor grade, final pathological stage and MVD in biopsies and prostatectomies, the median value, i.e. 48 of microvessel counts of 61 tumors was set as the cut-off point. The $\chi 2$ test, MANOVA, discriminative analysis, Pearson coefficient (χ) multiple correlation coefficient (R) were applied to identify the associations between MVD counts of 48 and less of 48 and more than 48 and final pathologic results, using a significance level of 0.05, and level of 0.001 for a very high statistical significance.

Results

Clinical findings

The mean age was 66 years (SD ± 5.28; range from 52 to 78) at the time of surgery. The pretreatment serum PSA ranged from 2.8 to 73.3 ng/mL (mean 14.73 ± 12.75 ng/mL) (Table 1). PSA (continuous variable) was examined for the association with pT (MANOVA: $p = 0.817$, that is χ^2 test: $p = 0.602$) and GS (MANOVA: $p = 0.901$, that is $\chi 2$ test: $p = 0.949$) both as a continuous variable and in a categorical fashion (< 10 ng/mL *vs* 10–20 ng/mL *vs* > 20 ng/mL). No statistically significant association was seen (Tables 2 and 3).

Table 1

Clinical and pathological parameters in prostate carcinoma in 61 patients with prostate cancer

Variables	Patients (n = 61)	
	n	%
Tumor stage		
T2	37	60.66
T3	24	39.34
Metastasis in regional lymph nodes		
N0	55	90.16
N1	6	9.84
Gleason score (GS)		
< 7	21	34.43
≥ 7	40	65.57

Table 2

Tumor stage (pT) and clinical and pathological results in prostate carcinoma

Variables	pT2 (n = 37)	pT3 (n = 24)	Significance
Age at surgery (years), mean ± SD, (range)	66.03 ± 5.84 (52–75)	66.92 (SD ± 4.35) (54–78)	$p = 0.525$ MANOVA; $p > 0.1$
Preoperative sPSA, (ng/mL) mean ± SD, (range)	16.13 ± 13.98 (2.8–73.3)	15.27 (SD ± 14.49) (6.1–70.0)	$p = 0.817$ MANOVA; $p > 0.1$
< 10, n (%)	12 (32.4)	10 (41.7)	$p = 0.602$ χ^2–test; $p > 0.1$
10–20, n (%)	17 (45.9)	11 (45.8)	
> 20 n, (%)	8 (21.6)	3 (12.5)	
Gleason score (GS), n (%)			
< 7	20 (54.1)	17 (45.9)	$p = 0.000$ χ^2–test; $p < 0.001$
≥ 7	1 (4.2)	23 (95.8)	
Metastasis in regional lymph nodes pN, n (%)			
N0	37 (60.65)	18 (29.50)	$p = 0.001$ χ^2– test; $p = 0.001$
N1	0	6 (9.84)	

PSA – prostate specific antigen.

Table 3

Gleason score (GS) and clinical and pathological results in prostate carcinoma

Variables	Gleason score		Significance
	< 7	≥ 7	
Age at surgery (years), mean ± SD (range)	66.19 ± 6.10) (52–75)	66.47 ± 4.88 (54–78)	$p = 0.844$ MANOVA; $p > 0.1$
Preoperative sPSA (ng/mL) mean ± SD (range)	15.48 ± 11.11 (4.1–47.8)	15.95 ± 15.52 (2.8–73.3)	$p = 0.901$ MANOVA; $p > 0.1$
< 10, n (%)	7 (31.8)	15 (68.2)	$p = 0.949$ χ^2– test; $p > 0.1$
10–20, n (%)	10 (35.7)	18 (64.3)	
> 20, n (%)	4 (36.4)	7 (63.6)	
Tumor stage (pT), n (%)			
T2	20 (54.1)	17 (45.9)	$p = 0.000$ χ^2– test; $p < 0.001$
T3	1 (4.2)	23 (95.8)	
Metastasis in regional lymph nodes (pN), n (%)			
N0	21 (38.2)	34 (61.8)	$p = 0.062$ χ^2– test; $p < 0.1$
N1	0	6 (100)	

Pathological results

Final pathological staging in RP of 61 tumors fulfilling the selection criteria recorded 37 (61%) as pT2, and 24 (39%) as pT3 (organ-extended) (Table 1). The 24 pT3 cases demonstrated EPE in all the cases of which seminal vesicles invasion in 19 (31%). These scores correlated significantly with tumor stage when analyzed by the χ^2 test ($p < 0.0001$ for biopsies and prostatectomies, respectively) as shown in Table 2. Regional lymph node metastases were present in 6 (10%) cases, all of them were pT3 and GS ≥ 7 (GS 7–2 cases, GS 8–3 patients and GS 9–1 case). A statistically significant correlation was found between metatases in regional lymph nodes on one side and pT ($p = 0.001$; χ^2 test), and GS ($p = 0.062$ χ^2 test) (Tables 2 and 3) on the other side. The median Gleason score for all tumors was 6 (range 4–8) in core biopsies and 7 (range 4–9) in prostatectomies. There was a significant discordance between biopsy and matched prostatectomy grades. Needle core biopsy underestimated tumor grade in 39% of cases (15 cases: GS6→GS7; 4 cases: GS7→GS8; 2 patients: GS6→GS8; 2 cases: GS5→GS6; and 1 patient in GS5→GS7, GS4→GS6, GS 7→GS 9) and overestimated in 1% (1 case: GS 8→GS 7). pT2 tumors scored 6 (range 4–8) in biopsies and 7 (range 4–9) in RP; the median score in carcinomas staged as pT3 was 7 (range 4–8) in core biopsies and in RP (range 4–9). These scores correlated significantly with the tumor stage when analyzed by the χ^2 test ($p < 0.0001$ for biopsies and prostatectomies, respectively) as shown in Tables 2 and 3. The median number of biopsies *per* case involved by cancer was 3 (range 1–5). Immunostaining for CD31 exhibited intense and homogeneous staining of the endothelial cells of blood microvessels on all the 61 examined cases, as evidenced by positive staining of non-tumor-associated vessels (Figures 1 and 2). It did not react with lymphatic endothelium or fibroblasts. Immunoreactivity was also observed in a small number of macrophages and plasma cells. MVD ranged from 22 to 89 (mean 49.84 ± 13.36) in core biopsies and 46.85 ± 14.47 in prostatectomies). When comparing these values, no statistical significance was found (MANOVA: $p = 0.239$). MVD (continuous variable) was examined for the association with pT both as a continuous variable and in a categorical fashion (pT2 *vs* pT3).

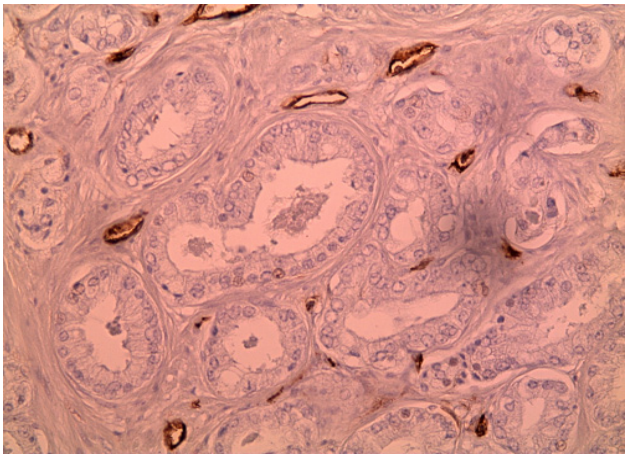

Fig. 1 – Prostate carcinoma showing low vascularization, Gleason score 6 (CD31; × 200).

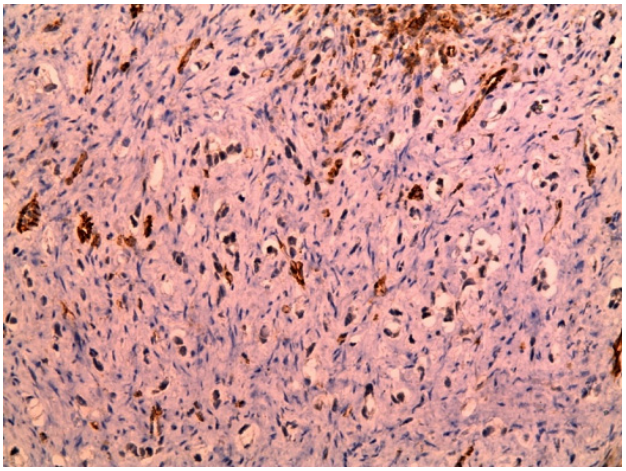

Fig. 2 – Prostate carcinoma showing high vascularization, Gleason score 9 (CD31; × 200).

A very high statistical significance was evident when the microvessel density in prostatectomies and the final pathological stage were compared (MANOVA: $p = 0.001$) (Table 4, Figure 3).

Table 4

Microvessel density (MVD) and clinical and pathological results in prostate carcinoma

Parameters	Patients (n)	MVD ± SD	MVD ≤ 48 (n = 24)	MVD > 48 (n = 37)	*p* value
Age at surgery (years), mean ± SD (range)			66.96 ± 5.34 (52.0–74.0)	66.00 ± 5.29 (54.0–78.0)	$p = 0.493$ MANOVA; $p > 0.1$
Preoperative sPSA (ng/mL), mean ± SD (range)			18.55 ± 15.59 (3.4–73.3)	14.00 ± 12.89 (2.8–70.0)	$p = 0.220$ MANOVA. $p > 0.1$
< 10	22	53.64 ± 12.51 (30–78) (NB) 47.14 ± 14.82 (30–81) (RP)	6 (25.0%)	16 (43.2%)	
10–20	28	48.29 ± 13.52 (25–72) (NB) 45.71 ± 13.28 (28–89) (RP)	12 (50.0%)	16 (43.2%)	$p = 0.279$ χ^2-test; $p > 0.1$
> 20	11	46.18 ± 13.97 (22–72) (NB) 49.18 ± 17.59 (29–88) (RP)	6 (25.0%)	5 (13.5%)	
p-value (MANOVA)		$p = 0.393$; $p > 0.1$			
Gleason score. GS mean ± SD (range)					
< 7	21	46.11 ± 13.51 (22–73) (NB) 38.79 ± 9.54 (28–60) (RP)	12 (50.0%)	9 (24.3%)	$p = 0.039$ χ^2-test; $p < 0.05$
≥ 7	40	53.00 ± 12.57 (27–78) (NB) 53.70 ± 14.51 (29–89) (RP)	12 (50.0%)	28 (75.7%)	
p-value (MANOVA)		$p = 0.044$; $p < 0.1$			
Tumor stage. pT mean ± SD (range)					
T2	37	46.24 ± 13.10 (22–73) (NB) 42.24 ± 11.64 (28–70) (RP)	19 (79.2%)	18 (48.6%)	$p = 0.017$ χ^2-test; $p < 0.05$
T3	24	55.38 ± 11.99 (27–78)(NB) 53.96 ± 15.73 (32–89) (RP)	5 (20.8%)	19 (51.4%)	
p value (MANOVA)		$p = 0.001$; $p = 0.001$			
Metastasis in regional lymph nodes. (pN) mean ± SD (range)					
N0	55	48.85 ± 13.63 (22–78) (NB) 45.60 ± 13.79 (28–89) (RP)	24 (100%)	31 (83.8%)	$p = 0.038$ χ^2-test; $p < 0.05$
N1	6	58.83 ± 5.19 (50–65) (NB) 58.33 ± 16.83 (39–88) (RP)	0 (0%)	6 (16.2%)	
p-value (MANOVA)		$p = 0.071$; $p = 0.082$ (NB). $p = 0.040$ (RP); $p < 0.1$			

NB – needle biopsy; RP – radical prostatectomy.

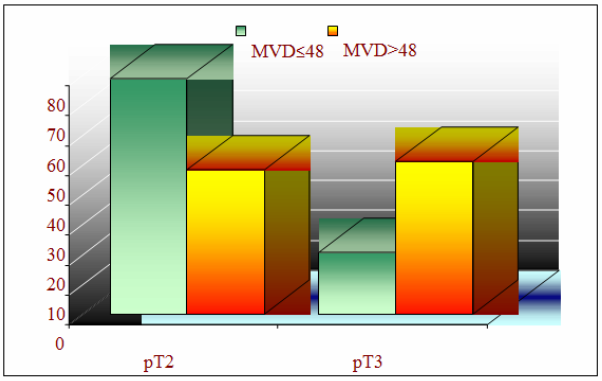

Fig. 3 – Correlation between pathological stages (pT2 *vs* pT3) and microvessel density (MVD).

This difference in MVD between pT2 and pT3 tumors was confirmed by univariate analysis when the mean microvessel count of all tumors, *ie*, 48, was set as a cut-off value (χ^2: $p = 0.017$) (Figure 3, Table 4). A statistically significant correlation was found between metatases in regional lymph nodes (pN0 *vs* pN1) and MVD (MANOVA; $p = 0.071$ for continous and χ^2: $p = 0.038$ for categorical variable) (Table 4). MVD was examined for association with GS both as a continuous variable and in a categorical fashion (≤ 48 *vs* > 48). Although a statistically significant discrimination between low- and high-grade tumors was found (MANOVA: $p = 0.044$ for continuous and χ^2 test: $p = 0.039$ for categorical variable), it did not reach the level of final pathological stage. An increase in MVD was not associated with pretreatment PSA as a continous variable (MANOVA: $p = 0.393$) or as a categorical variable (χ^2 test : $p = 0.279$) (Table 4). All those variables previously analyzed (clinical and pathological characteristics) were statistically significantly associated with final pathological stage (pT). Additionally, univariate analyses (Pearson test, multiple correlation coefficient) and multivariate analyses (MANOVA; discriminative analysis) were done in order to determine the most significant factors in prediction organ-confined (pT2) *versus* organ-extending prostate cancer (pT3). The Gleason score ($p < 0.001$) and metastases in regional lymph nodes ($p = 0.001$) showed a strong statistical significance. MVD also showed statistical significance ($p = 0.017$), but not so strong as previously mentioned (Table 5). Preoperative serum PSA alone was not significant in predicting final pathological stage ($p = 0.322$) (Table 6).

Table 5

Microvessel density (MVD) studies: correlation with pathological results

Study	Specimen	IHC/Method	Correlation/Yes	Correlation/ No
Weidner et al. [35]	74 RP	F 8/Weidner	GS, pN1	
Brawer et al. [24]	32 RP and 5 TURP	F 8/computer	pT	
Silberman et al. [41]	109 RP (GS5-7)	CD31/Weidner modified x 400		GS, EPE
Rubin et al. [25]	87 RP	CD31/Weidner		GS, pT
Rogatsch et al. [18]	36 NB and RP	CD31/Weidner modified × 400	pT	
Offersen et al. [36]	64 TURP	CD31, F8/ -	Survival	
Borre et al. [38]	221 NB TURP	F8/ -	GS, clinical stage	
Bostwick et al. [21]	186 NB and RP	F8/computer	pT	
Di Lorenzo et al. [4]	72 RP	CD31/Weidner	pT, GS, sPSA	
Tretiakova et al. [48]	169 RP	CD31/computer		GS
Taverna et al. [49]	27 RP	CD34/computer	sPSA	pT, GS
Erbersdobler et al. [43]	3261 RP	CD31/Weidner	pT, GS	sPSA
van Niekerk et al. [47]	28 RP	CD31/computer		GS
Steiner et al. [15]	69 RP	CD31,CD34 /Weidner × 400	pT, GS	
Jiang et al. [9]	73 RP	CD31/Weidner × 400	GS	sPSA

IHC – immnunohistochemistry; RP – radical prostatectomy; TURP – transurethral *resectio prostatae*; NB – neobladder; SPSA – serum prostate-specific antigen; GS – Gleason score; EPE – extraprostatic extension.

Table 6

Correlation between pathological stages (pT2 vs pT3) and clinical and pathological variables and microvessel density (MVD)

Variables	χ	R	F	*p*	c.dsc
Preoperative sPSA (<10 *vs* 10–20 *vs* > 20 ng/mL)	0.128	0.129	0.999	0.322	0.027
MVD (≤ 48 *vs* > 48)	0.292	0.305	6.059	0.017	0.012
Gleason score (< 7 *vs* ≥ 7)	0.456	0.513	21.065	0.000	0.237
pN (pN0 *vs* pN1)	0.379	0.410	11.929	0.001	0.183

Pearson's contigency coefficient (χ); multiple correlation coefficient (R); discrimination coefficient (c.dsc).

Discussion

If prostate cancer is diagnosed on time, whether primary or recurrent, it may be curatively treated. The process of the very diagnostics, screening and staging of the disease is controversial, due to limitation in its disclosure [14]. As a result of widespread testing of patients for sPSA over the past decade, most patients with prostate cancer now present with the clinically localized disease, and their tumors are rarely graded with Gleason scores < 6 [19]. In general, serum PSA levels correlate with a larger tumor volume, advanced pathologic stage and higher grade [20–22]. Although higher grade cancer produces less PSA per cell, when compared to lower grade tumors, overall, poorly differentiated tumors are associated with higher PSA levels as these tumors tend to be larger and of a more advanced stage [23]. There are exceptions with very high grade prostate cancers which are so poorly differentiated that associated serum PSA levels are disproportionally low [20]. In our study, a statistically significant correlation was not found between serum PSA levels (as continuous and categorical variables) and stage of disease (pT2 *vs* pT3), and also between sPSA and tumor grade (Gleason score) (<7 *vs* ≥ 7). Preoperative serum PSA in our investigation, as in some other studies, showed that it could not give useful pathologic correlations on individual basis, for each patient [5, 9, 22, 24–26]. A significant overlap of sPSA values between different tumor stages (pT2 *vs* pT3) did not enable clear distinction between these values (regarding organ-confined prostate carcinoma *versus* organ-extending carcinoma) in relation to locally limited PC, namely locally invasive PC.

Clinical doctors classify patients with newly diagnosed PC by stage and grade. This classification is important because of the extraordinary variability in the potential for disease progression. Tumor grade, stage, and the presence of competing medical hazards are the most powerful predictors of survival [19]. There is a significant discordance between biopsy and matched prostatectomy grades. Needle core biopsy underestimates tumor grade in 33–45% of cases and overestimates in 4–32% [27–29]. Grading errors are common in biopsies with small amounts of tumor and low-grade tumor [30]. In our study, Gleason score values before and after the surgery differed in 40.98% of the patients. Needle core biopsy underestimated tumor grade in 39.34% of the cases, with the greatest discordance in distinguishing GS 6 from GS 7. Needle core biopsy overestimated tumor grade in one patient 81.63% (GS 8 *versus* GS 7 on matched prostatectomy). Judging by univariate and multivariate studies, tumor grade is one of strongest and most useful prognostic parameters which forecast tumor stage [20, 27, 31]. This possibility of forecasting can be applied to almost every determination of pathologic tumor stage, including EPE (extraprostatic extension), SVI (seminal vesicle invasion), regional node metastasis, and bone metastasis. In research, patients are usually grouped according to the Gleason score as low risk (GS < 7), medium-risk (GS 7) and high-risk groups (GS 8–10). In some studies, patients with the Gleason score 7 have the same disease outcome and behavior of PC as the ones with GS 8–10, so some researchers put them in the same group [18]. According to some researchers, GS ≥ 8 determined on needle core biopsy as a strong prognostic factor which indicates the possibility of the existence of regional lymph node metastasis [32]. Both sPSA and GS can provide significant prognostic data when their values are at either high (sPSA < 20 ng/mL, GS 8–10), or low (sPSA < 4 ng/mL, GS 2–4) level. However, the majority of patients are exactly in the middle, namely with GS 7 and intermediary level sPSA [22]. Our study shows a statistically high correlation between tumor stage and grade, namely 95.8% of patients with GS ≥ 7 had locally invasive PC (pT3). Also, all the patients with regional lymph nodes metastases (pN1) had poorly differentiated PC (GS ≥ 7). A need for correct preoperative tumor stage determination is essential, especially after studies on massive tumor series clinically diagnosed as organ-confined prostate cancer (T2), out of which approximately 24–60% after RP had pathologic confirmation of locally invasive and metastatically spread disease (pT3, pN1) (1.3–8). In 8–45% patients PC were preoperatively clinically over-staged [9]. In our research, after radical prostatectomy, 61% PC were in tumor stage pT2, and 39% were in a stage pT3, while as 10% patients had metastatically spread disease.

Since 1971, when Folkman determined that tumor growth and dissemination depended on angiogenesis and also that tumors, along with inflammatory cells and related vasculature, created a complex ecosystem which communicates through chemical signals, many studies have been made in order to support this theory [33]. MVD varies widely depending on tumor type; all tumors, including the ones with smallest MVD, depend on angiogenesis [34].

Reference publications contain numerous conflicting studies which relate to the possibility of angiogenesis to predict pathological stadium for patients with clinically organ-confined prostate carcinoma. Many studies proved the connection between MVD and GS, disease stage, as well as possibilities of metastatic spread of carcinoma in future, while as in other studies MVD in relation to stage pT has not shown superior predictive value (Table 6). Still, most researchers estimate that MVD increased values have a prognostic significance in estimation of biological behavior of PC. In a Weidner et al. [35] research, patients with metastasis had double higher values of MVD in relation to locally invasive PC, and higher values of MVD were related to higher Gleason score, but only in poorly differentiated PC. Offersen et al. [36] points to the fact that the maximum value, and not the median one MVD, is significantly associated with survival estimation for patients with CP. In a Bostwick-lead multi-institutional study, logOMVD (optimized microvessel density) was statistically significantly correlated with GS and pre-operative sPSA values, as well as with pT3. However, disease outcome forecasted by OMVD did not relate to patients who had organ-confined disease (pT2) and GS 6–9 [21]. In our research, higher MVD continual and categorical variables were correlated with poorly differentiated prostate carcinoma, namely GS ≥ 7, with higher tumor stage (pT3), namely they were higher in metastatic spread disease (pN1). Such results have been confirmed in studies of many au-

thors [4, 6, 18, 37–41]. Our study does not show a statistically significant correlation with pre-operative values of sPSA, which is in line with certain findings listed in the literature [5, 9, 25, 42]. In a tissue microarray study (TMA), used on the largest number of samples so far (3261 RP), Erbersdobler et al. [43] prove using univariate analyses, a significant correlation between an increased MVD and advanced stadium of PC, pT3 ($p < 0.001$), as well as a higher GS, (GS $\geq$ 7) ($p < 0.001$), but also points out to the existence of significant differences between tumors, taking into account their localization, that is, that the transitional zone tumors (TZ) have a lower MVD, compared to the peripheral zone (PZ) tumors. However, MVD has not been proved to be an independent prognostic parameter in multivariant analyses, instead it is closely connected to the other factors contributing to tumor aggression. The authors point out that if the antiangiogenic therapy for prostate cancers has not been established yet and if it starts being applied, knowing the differences in MVD between individual tumors and tumor locations (TZ *versus* PZ tumors) would become significant [43]. Steiner et al. [15] has established a mild correlation between mRNA of individual endothelial factors (CD31, CD 34) in prostate cancer tissue compared to the histologically determined MVD, even though higher values of histologically determined MVD were statistically significantly related to higher GS and stadium, pT3 ($p < 0.001$).

Contrary to the previously reported research results, certain authors have reached completely opposite conclusions in their studies [41, 44]. Silberman et al. [41] determined a correlation between MVD and tumor progression after RP, but not with pathologic stage in patients who had GS 5–7. A correlation between MVD and disease stage, as well as metastasis, was not proved in the work of Matsushima et al. [45], while a correlation between MVD and Gleason grade was statistically almost significant. Rubin et al. [25] also did not find a correlation between MVD and GS, tumor stage, positive surgical margins or seminal vesicle invasion, but also not with increased postoperative sPSA values as a sign of disease recurrence. By using multivariate analysis (using estimation p53, retinoblastoma, chromogranin A and MVD), Krupski et al. [46] has determined that MVD values showed no prognostic significance of importance in comparison to p53 and retinoblastoma in estimation of patient survival. Gettman et al. [5] found no correlation between OMVD and DNA ploidy, Gleason grade, pathologic stage, or with sPSA (preoperative serum PSA), neither the application of univariate and multivariate analysis proved OMVD as a predictor of clinical or biochemical disease recurrence. By using the image analysis system for determination MVD, van Niekerk et al. [47] determined no consistent increase of MVD in TZ tumors in terms of the surrounding unchanged tissue of prostate and benign tissue hyperplasia, unlike PZ prostate cancer, which had almost double increase of MVD value, explaining this with intrinsic biological differences between these two zonal types of tumors (such as heterogeneous of microvasculature of TZ tumor), which, at least partly, condition their different biological behavior. In this study, no correlation was found between MVD and Gleason score of TZ and PZ tumor. Unlike van Niekerk et al, Tretiakova et al. [48] in their research using computer analysis of MVD conclude that MVD is not statistically significantly increased in PC compared to the normal surrounding tissue of the prostate, as well as neither in low grade PC (GS $\leq$ 3 + 4) compared to high grade PC (GS $\geq$ 4 + 3), as well as that MVD cannot be considered a useful prognostic parameter. Taverna et al. [49, 50], examining two-dimensional geometrical complexity of vasculature of PC, divided the patients in two groups, taking into account increase/decrease of fractal dimension of tumorous vascular surface and surrounding non-tumorous tissue, establishing that the patients with a lower tumorous vascular surface had a worse clinical outcome, that is, that the tumor progression was not dependant on angiogenesis. At the end, Taverna et al. [51] leave an open question as to whether angiogenesis is a "canonic hallmark" of PC and point out that there are no powerful methods of quantifying the reversal of neovascularity.

The majority of these studies, using different antibodies, methods of counting and selection, show some significant correlations between MVD and poorly differentiated PC and shorter patient survival, suggesting that MVD is a strong measurer of tumor angiogenic activity. The controversy of results is a consequence of practical problems which limit the usage of MVD measuring on surgical material, namely there is no consensus neither on vessels counting nor on "cut-off"" value which would differentiate/separate high- and low-grade neoplasms. Different methodologic problems occur while counting blood vessels, such are different observations by different pathologists, even the same pathologist, during the first count/selection of areas with most intensive neovascularisation ('hot-spot'), as well as heterogeneity of tumor, which remain unresolved and can therefore influence on results of immunohistochemistry analysis. The number of published results on MVD up to now is 14–300, and along with that 'cut-off', which varies between 23–160 [35, 39, 43, 52, 53]. In our research the mean value of MVD on samples of needle core biopsies was 49.84 ± 13.36 (22–78), and on samples of matched RP 46.85 ± 14.47 (28–89), and after their comparison there was no statistically significant difference, while as 'cut–off' value was 48. If one neglects the variations in patient selection (for example, Tretiakova et al. [48] divide 67% of patients with GS 7 PC to low grade PC group ($\leq$ 3 + 4) and high grade PC group ($\geq$ 4 + 3), one finds that those different values are mostly conditioned by different techniques of tumor blood vessels counting. These differences can be a consequence of endothelial antibodies choice, selection of vascular parameters, choice of tumor field in which measurement is done, vessel counting method, determination of 'cut-off' values which is used in correlation analysis along with other clinic and pathologic variables and survival, as well as wrong statistical methods which are used. Selection of 'cut-off' values was based on personally estimated median value of MVD, bellow which the prognosis was good, and above it bad, and therefore it had to be seen arbitrary until valid values were identified. Such differences can depend on the fact whether MVD is estimated on periphery, or in the center of tumor [39]. As it was shown that there was a strong correlation in MVD values gained by using different antibod-

ies, where CD-31, which we used in our study, was shown to be more sensitive, showing 18–33% higher results of microvessel counting, MVD, than some other antibodies which are usually used (for example, CD34 is detectable, except in endothelial cells, in mesenchymal and inflammatory cells and lymphatic vessels), the biggest discrepancies related to other of the listed reasons [18, 45]. Studies that could not confirm the prognostic significance of MVD were the ones which mostly differed from the methodology described by Weidner et al. [16, 17, 35]. Each of the previously mentioned authors used numerous modifications of this method (e.g., determination of the so-called 'hot spots', namely areas with the highest number of blood vessels, how many fields are counted and where, whether focuses overlap). This study also used a modification of the Weidner [13] method of microvessel counting on needle biopsy and matched radical prostatectomy specimens on a high-power microscopic field, ×400. This method selection in our research has a foundation in certain studies on different tumors with good correlations between results of blood vessels calculation at ×400 magnification in relation to ×200 magnification [16]. The approach chosen in this investigation has also beensupported by several studies performed on other human tumor types showing good correlations between vascular counts performed in ×400 *versus* ×200 magnifications [13, 18]. MVD values in our study are higher in relation to previous studies [35, 53]. A possible explanation of such results is, as Rogatsch et al. [18] stressed, that higher resolution ×400 results in MVD value increase by 11–33% when it is compared to ×200 magnification as shown breast carcinoma [16, 17]. Application of image analysis system in histologically determined MVD, as was suggested by a few groups of researchers is more expensive, more demanding, unsuitable for routine application, and is not more accurate in comparison to calculation done by other researchers in person [5, 6, 21, 25, 47–49]. Precisely, these differences in the manner of determination of MVD in many studies, disable their adequate interpretation and comparison, thus a consensus-agreed methodology for determination of MVD could be used to provide more proper comparison and interpretation of MVD values when compared to clinical and pathological parameters.

Having in mind the values of GS and MVD, non-invasive imaging technique that can reflect both GS and MVD to be able to provide timely diagnostics and determination of PC characteristics [14]. Histological heterogeneity and multifocality of PC limit use of needle biopsy in determination of all carcinomas grades and sites [9]. What would be valuable for choosing targets for prostate biopsies would be an imaging method, which could indicate increasing in MVD and it could also provide a foreseeable Gleason score. This should result in a change of biopsy strategy, the outcome of which would be a higher detection rate of prostate cancer and a more accurate Gleason grading, meaning, a more adequate therapeutic strategy [9]. In line with this, the most used conventional imaging methods today are ultrasonography with moleculary targeted constrast microbubbles (CEUS) and magnetic resonance imaging (MRI), amended with moleculary, metabolic and functional imaging techniques [14]. Some researchers correlate the results obtained by imaging methods with histologically determined MVD. Lee et al. [54] evaluated tumorous angiogenesis using the mouse xenograft model injected with human PC-3 prostate cancer cells, using contrast-enhanced sonography, establishing a statistically significant correlation of the US maximum intensity and CD31-positive microvesel count. Jiang et al. [9] established on samples of needle prostate biopsies, that the peak intensity of prostate cancer at CEUS was statistically significantly increased with a higher GS and histologically determined MVD. Osimani et al. [55] showed that in PC blood volume and permeability surface area product measurements obtained with perfusion computed tomography had the highest correlation with immunohistochemical markers of angiogenesis, MVD [55]. Unlike them, Franiel et al. [56], using MRI perfusion and blood volume hotspots with histological MVD, determined no significant correlation, explaining this with heterogeneous vascularization of the normal and tumorous prostate tissues, as well as different thickness of MRI slices, that is, histological paraffin blocks, but also with technical limitations of MRI, suggesting that the computer-based 3D prostate model could be used in the future to provide a more accurate correlation of histological and MRI imaging findings. Even though the prognostic value of microvessel density in prostate cancer is contradictory and microvessel density is not recommended for routine application by the World Health Organization, it is still the subject of research, particularly in the samples of needle prostate biopsies, where its prognostic significance is still unclear [57].

Conclusion

Although the number of patients in this study was small, the obtained results indicate that quantification of microvascular density in biopsies is an accurate pre-operative predictor of tumor stage, discriminating between organ-confined and organ-extending neoplasms.

REFERENCES

1. *Partin AW, Kattan MW, Subong EN, Walsh PC, Wojno KJ, Oesterling JE,* et al. Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. JAMA 1997; 277(18): 1445−51.
2. *Oesterling JE*. Prostate specific antigen: a critical assesment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 1991; 145(5): 907−32.
3. *Epstein JI, Walsh PC, Carmichael M, Brendler CB*. Pathologic and clinical findings to predict tumor extent of nonpalpable (stage T1c) prostate cancer. JAMA 1994; 271(5): 368−74.
4. *Di Lorenzo G, De Placido S, Autorino R, De Laurentiis M, Mignogna C, D'Armiento M,*. Expression of biomarkers modulating prostate cancer progression: implications in the treatment of the disease. Prostate Cancer Prostatic Dis 2005;8(1):54-59.

5. *Gettman MT, Bergstralh EJ, Blute M, Zincke H, Bostwick DG.* Prediction of patient outcome in pathologic stage T2 adenocarcinoma of the prostate: lack of significance for microvessel density analysis. Urology 1998; 51(1): 79−85.
6. *Mucci LA, Powolny A, Giovannucci E, Liao Z, Kenfield SA, Shen R,* et al. Prospective study of prostate tumor angiogenesis and cancer-specific mortality in the health professionals follow-up study. J Clin Oncol 2009; 27(33): 5627−33.
7. *Partin AW, Yoo J, Carter HB, Pearson JD, Chan DW, Epstein JI*, et al. The use of prostate specific antigen, clinical stage and Gleason score to predict pathological stage in men with localized prostate cancer. J Urol 1993; 150(1): 110−4.
8. *Walsh PC, Partin AW, Epstein JI.* Cancer control and quality of life following anatomical radical retropubic prostatectomy: results at 10 years. J Urol 1994; 152(5 Pt 2): 1831−6.
9. *Jiang J, Chen Y, Zhu Y, Yao X, Qi J.* Contrast enhanced ultrasonography for the detection and characterisation of prostate cancer: correlation with microvessel density and Gleason score. Clin Radiol 2011; 66(8): 732−7.
10. *Zetter BR.* Angiogenesis and tumor metastasis. Annu Rev Med 1998; 49: 407−24.
11. *Nagy JA, Dvorak AM, Dvorak HF.* Vascular hyperpermeability, angiogenesis, and stroma generation. Cold Spring Harb Perspect Med 2012; 2(2): a006544.
12. *Eberhard A, Kahlert S, Goede V, Hemmerlein B, Plate KH, Augustin HG.* Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for anti-angiogenic tumor therapies. Cancer Res 2000; 60(5): 1388−93.
13. *Weidner N.* Current pathologic methods for measuring intratumoral microvessel density within breast carcinoma and other solid tumors. Breast Cancer Res Treat 1995; 36(2): 169−80.
14. *Russo G, Mischi M, Scheepens W, De la Rosette JJ, Wijkstra H.* Angiogenesis in prostate cancer: onset, progression and imaging. BJU Int 2012; 110(11 Pt C): E794−808.
15. *Steiner I, Jung K, Miller K, Stephan C, Erbersdobler A.* Expression of endothelial factors in prostate cancer: a possible role of caveolin-1 for tumour progression. Oncol Rep 2012; 27(2): 389−95.
16. *Weidner N, Semple JP, Welch WR, Folkman J.* Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 1991; 324(1): 1−8.
17. *Weidner N, Folkmann J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S,* et al. Tumor angiogenesis: a new significant and independent prognostic factor in early-stage breast carcinoma. J Natl Cancer Inst 1992; 84(24): 1875−87.
18. *Rogatsch H, Hittmair A, Reissigl A, Mikuz G, Feichtinger H.* Microvessel density in core biopsies of prostatic adenocarcinoma: a stage predictor?. J Pathol 1997; 182(2): 205−10.
19. *Albertsen PC, Hanley JA, Barrows GH, Penson DF, Kowalczyk PD, Sanders MM*, et al. Prostate cancer and the Will Rogers phenomenon. J Natl Cancer Inst 2005; 97(17): 1248−53.
20. *Epstein JI, Netto GJ.* Prostate biopsy interpretation. 4th ed. Philadelphia, PA: Lippincott Williams Wilkins; 2008.
21. *Bostwick DG, Wheeler TM, Blute M, Barrett DM, MacLennan GT, Sebo TJ*, et al. Optimized microvessel density analysis improves prediction of cancer stage from prostate needle biopsies. Urology 1996; 48(1): 47−57.
22. *Kuniyasu H, Troncoso P, Johnston D, Bucana CD, Tahara E, Fidler IJ,* et al. Relative expression of type IV collagenase, E-cadherin, and vascular endothelial growth factor/vascular permeability factor in prostatectomy specimens distinguishes organ confined from pathologically advanced prostate cancers. Clin Cancer Res 2000; 6(6): 2295−308.
23. *Partin AW, Carter HB, Chan DW, Epstein JI, Oesterling JE, Rock RC*, et al. Prostate specific antigen in the staging of localized prostate cancer: influence of tumor differentiation, tumor volume and benign hyperplasia. J Urol 1990; 143(4): 747−52.
24. *Brawer MK, Deering RE, Brown M, Preston SD, Bigler SA.* Predictors of pathologic stage in prostatic carcinoma: the role of neovascularity. Cancer 1994; 73(3): 678−87.
25. *Rubin MA, Buyyounouski M, Bagiella E, Sharir S, Neugut A,* et al. Microvessel density in prostate cancer: lack of correlation with tumor grade, pathologic stage and clinical outcome. Urology 1999; 53(3): 542−47.
26. *Daris B, Munda M, Hajdinjak T, Kavalar R, Štiblar-Martičić D.* Comparison of microvessel density and PSA level in prostatic adenocarcinoma of Gleason score 6 and 7. Acta Medico Biotech 2012; 5(1): 54−60.
27. *Bostwick DG, Meiers I.* Neoplasms of the prostate. In: *Bostwick DG, Cheng L*, editors. Urologic surgical pathology. 2nd. Edinburgh: Mosby Elsevier. 2008. p. 443−580.
28. *Tomioka S, Nakatsu H, Suzuki N, Murakami S, Matsuzaki O, Shimazaki J.* Comparison of Gleason grade and score between preoperative biopsy and prostatectomy specimens in prostate cancer. Int J Urol 2006; 13(5): 555−9.
29. *King CR, McNeal JE, Gill H, Brooks JD, Srinivas S, Presti JC Jr.* Reliability of small amounts of cancer in prostate biopsies to reveal pathologic grade. Urology 2006; 67(6): 1229−34.
30. *Versalainen S, Lipponen P, Talja M, Alhava E, Syrjanen K.* Tumor vascularity and basement membrane structure as prognostic factors in T1-2M0 prostatic adenocarcinoma. Anticancer Res 1994;14: 709−14.
31. *Haese A, Vaisanen V, Lilja H, Kattan M, Rittenhouse H, Pettersson K,* et al. Comparison of predictive accuracy for pathologically organ confined clinical stage T1c prostate cancer using human glandular kallikrein 2 and prostate specific antigen combined with clinical stage and Gleason grade. J Urol 2005; 173(3): 752−6.
32. *Bluestein DL, Bostwick DG, Bergstralh EJ, Oesterling JE.* Eliminating the need for bilateral pelvic lymphadenectomy in select patients with prostate cancer. J Urol 1994; 151(5): 1315−20.
33. *Folkman J, Shing Y.* Angiogenesis. J Biol Chem 1992; 267(16): 10931−4.
34. *Ferrer FA, Miller LJ, Andrawis RI, Kurtzman SH, Albertsen PC, Laudone VP,* et al. Angiogenesis and prostate cancer: In vivo and in vitro expression of angiogenesis factors by prostate cancer cells. Urology 1998; 51(1): 161−7.
35. *Weidner N, Caroll PR, Flax J, Blumenfeld W, Folkman J.* Tumor angiogenesis correlates with metastasis in invasive prostate cancer. Am J Pathol 1993; 143(2): 401−9.
36. *Offersen BV, Borre M, Overgaard J.* Immunohisto-chemical determination of tumor angiogenesis measured by the maximal microvessel density in human prostate cancer. APMIS 1998; 106(4): 463−9.
37. *Pallares J, Rojo F, Iriarte J, Morote J, Armadans LI, de Torres I.* Study of microvessel density and the expression of the angiogenic factors VEGF, bFGF and the receptors Flt-1 and FLK-1 in benign, premalignant and malignant prostate tissues. Histol Histopathol 2006; 21(8): 857−65.
38. *Borre M, Nerstrøm B, Overgaard J.* Association between immunohistochemical expression of vascular endothelial growth factor (VEGF), VEGF-expressing neuroendocrine-differentiated tumor cells, and outcome in prostate cancer patients subjected to watchful waiting. Clin Cancer Res 2000; 6(8): 1882−90.
39. *Bono AV, Celato N, Cova V, Salvadore M, Chinetti S, Novario R.* Microvessel density in prostate carcinoma. Prostate Cancer Prostatic Dis 2002; 5(2): 123−7.
40. *Arakawa A, Soh S, Chakraborty S, Scardino PT, Wheeler TM.* Prognostic significance of angiogenesis in clinically localized prostate cancer (staining for Factor VIII-related antigen and CD34 Antigen. Prostate Cancer Prostatic Dis 1997; 1(1): 32−8.
41. *Silberman MA, Partin AW, Veltri RW, Epstein JI.* Tumor angiogenesis correlates with progression after radical prostatectomy

but not with pathologic stage in gleason sum 5 to 7 adenocarcinoma of the prostate. Cancer 1997; 79(4): 772−9.

42. *Tsui KH, Shen BY, Sun GH, Lee SH, Chang PL*. Probability based diagnostic biopsy specimens as predictors of tumor grade and stage found. Arch Androl 2004; 50(5): 333−7.
43. *Erbersdobler A, Isbarn H, Dix K, Steiner I, Schlomm T, Mirlacher M,* et al. Prognostic value of microvessel density in prostate cancer: a tissue micro array study. World J Urol 2010; 28(6): 687−92.
44. *Gyftopoulos K, Vourda K, Sakellaropoulos G, Perimenis P, Athanasopoulos A, Papadaki E*. The angiogenic switch for vascular endothelial growth factor-A and cyclooxygenase-2 in prostate carcinoma: correlation with microvessel density, androgen receptor content and Gleason grade. Urol Int 2011; 87(4): 464−9.
45. *Matsushima H, Goto T, Hosaka Y, Kitamura T, Kawabe K*. Correlation between proliferation, apoptosis, and angiogenesis in prostate carcinoma and their relation to androgen ablation. Cancer 1999; 85(8): 1822−7.
46. *Krupski T, Harding MA, Herce ME, Gulding KM, Stoler MH, Theodorescu D*. The role of vascular endothelial growth factor in the tissue specific in vivo growth of prostate cancer cells. Growth Factors 2001; 18(4): 287−302.
47. *van Niekerk CG, Witjes JA, Barentsz JO, van der Laak JA, Hulsbergen-van de Kaa CA*. Microvascularity in transition zone prostate tumors resembles normal prostatic tissue. Prostate 2013; 73(5): 467−75.
48. *Tretiakova M, Antic T, Binder D, Kocherginsky M*. Microvessel density is not increased in prostate cancer: digital imaging of routine sections and tissue microarrays. Hum Pathol 2013; 44(4): 495−502.
49. *Taverna G, Colombo P, Grizzi F, Franceschini B, Ceva-Grimaldi G, Seveso M,* et al. Fractal analysis of two-dimensional vascularity in primary prostate cancer and surrounding non-tumoral parenchyma. Pathol Res Pract 2009; 205(7): 438−44.
50. *Taverna G, Grizzi F, Colombo P, Graziotti PP*. Microvessel density estimate: friendorfoe in the light of prostate vascular system complexity. World J Urol 2010; 28(3): 405−6.
51. *Taverna G, Grizzi F, Colombo P, Grazzioti P*. Is angiogenesis a hallmark of prostate cancer. Front Oncol 2013; 3(15): 1−3.
52. *Hlatky L, Hahnfeldt P, Folkman J*. Clinical application of antiangiogenic therapy: microvessel density, what it does and doesn't tell us. J Natl Cancer Inst 2002; 94(12): 883−93.
53. *Vartanian RK, Weidner N*. Endothelial cell proliferation in prostatic carcinoma and prostatic hyperplasia: correlation with Gleason's score, microvessel density, and epithelial cell proliferation. Lab Invest 1995; 73(6): 844−50.
54. *Lee HJ, Hwang SI, Chung JH, Jeon JJ, Choi JH, Jung HS*. Evaluation of tumor angiogenesis in a mouse PC-3 prostate cancer model using dynamic contrast-enhanced sonography. J Ultrasound Med 2012; 31(8): 1223−31.
55. *Osimani M, Bellini D, di Cristofano C, Palleschi G, Petrozza V, Carbone A,* et al. Perfusion MDCT of prostate cancer: correlation of perfusion CT parameters and immunohistochemical markers of angiogenesis. AJR Am J Roentgenol 2012; 199(5): 1042−8.
56. *Franiel T, Lüdemann L, Rudolph B, Lutterbeck E, Hamm B, Beyersdorff D*. Differentiation of prostate cancer from normal prostate tissue: role of hotspots in pharmacokinetic MRI and histologic evaluation. AJR Am J Roentgenol 2010; 194(3): 675−81.
57. *Bostwick DG, Foster CS*. Predictive factors in prostate cancer: current concepts from the 1999 College of American Pathologists Conference on Solid Tumor Prognostic Factors and the 1999 World Health Organization Second International Consultation on Prostate Cancer. Semin Urol Oncol 1999; 17(4): 222−72.

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